Chinese Agron. J. 1: 47-56 (1991)
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小孢子時期，蔗糖濃度及温度處理對麻竹 [*Sinocalamus latiflora* (Munro) McClure] 花葯培養胚狀體及癒合組織形成之影響

葉常青　許家言　蔡新聲

台灣省農業試驗所農藝系

摘要：麻竹〔*Sinocalamus latiflora* (Munro) McClure〕花葯培養於含N_6無機鹽類及MS有機成份，加上1 mg / l 2,4-D, 1 mg / l BA,0.2％活性碳，3～12％蔗糖及0.8％洋菜之培養基中能誘導出胚狀體及擬胚化癒合組織，並能再生得到單倍體植株(N＝36)。其中以含9％蔗糖濃度的培養基，胚狀體及癒合組織形成率最高。培養花葯之小孢子時期，則以含中單核期至早雙核期之花葯，胚狀體及癒合組織形成率較高。花序經10℃低温處理7天及花葯培養於30℃高温下，均能有效提高胚狀體及癒合組織之形成率。

關鍵語：花葯培養、麻竹、胚狀體、擬胚化癒合組織、單倍體植株。

Effects of microspore developmental stage, sucrose concentration and temperature treatment on embryoid and callus formation of bamboo [*Sinocalamus latiflora* (Munro) McClure] anther culture

C. C. Yeh, J. Y. Hsu and H. S. Tsay

Department of Agronomy, Taiwan Agricultural Research Institute, Taichung Taiwan 41301, Republic of China

Abstract. Embryoids and embryogenic callus could be induced from anthers of *Sinocalamus latiflora* (Munro) McClure cultured on medium containing N_6 inorganic salts and MS organic salts supplemented with 1 mg/l each of 2,4-D and BA, 0.2% charcoal, 3-12% sucrose and 0.8% Sigma agar. The derived embryoids and embryogenic callus could further differentiate into haploid plants (N = 36). Microspores at the developmental stage of mid-uninucleate to early-binucleate have been proved to be suitable for anther culture in terms of embryoid and embryogenic callus formation. Evaluation of medium composition indicated that sucrose concentration of 9% was most effective. Pretreatment of inflorescence at 10°C for 7 days before

dissecting anthers for culture also increased the percentage of anthers producing embryoids and callus. Furthermore, anthers cultured at 30°C resulted in higher rate of embryoid and callus initiation when compared with those at 25°C.

Key words: Anther culture, *Sinocalamus latiflora* (Munro) McClure, Embryoid, Haploid plant, Embryogenic callus

前　言

竹子屬於禾本科(Gramineae)，竹亞科(Bambusoideae)，常綠之單子葉植物，其分佈範圍遍及熱帶、亞熱帶甚至溫帶。成熟植株是建築、造紙上好的材料，且竹類的生長速度較其它木本植物快，更新迅速，成林後三、四年即可採伐利用，在天然資源漸趨匱乏的今日，為具發展潛力之加工原料。竹筍則是主要的蔬菜，近年來竹筍的外銷量已躍居我國農產品出口總值第一位，是目前最重要的經濟作物之一。

由於竹類一生只開一次花後即死亡，不規則的開花習性(12～120年)，加上種子存活率甚低，所以傳統雜交方法進行育種改良工作幾乎不可能(Nadgauda et al. 1990)。雖然曾有學者以竹子之其它培植體為材料進行組織培養，能經由器官形成或體胚發生的途徑成功的獲得再生植株(Hassan and Debergh 1987, Mehta et al. 1982, Rao et al. 1985, Yeh and Chang 1986a,b, 1987)。但竹子花葯培養至今仍無成功之實例。本研究係以麻竹〔*Sinocalamus latiflora* (Munro) McClure〕的花葯為材料，成功的獲得胚狀體及單倍體植株(證明係單倍體之資料已整理中，將於以後發表)，這個結果將能迅速獲得純系，而有助於竹子之育種。

材料與方法

一、花葯的消毒與接種

花葯培養所選用的材料是麻竹。由田間將整個花序採回，選取長度1～1.2cm的小穗，先以70%酒精消毒30秒、再以1%次氯酸鈉消毒10分鐘，然後以無菌水清洗3～4次。以接種針固定小穗基部，除去內外穎，每個小穗約有5～7朵小花，每朵小花有6個花葯，取其中一個進行時期檢定，另5個花葯接種於培養基中。低溫處理之材料將花序基部包裹濕潤衛生紙，裝入塑膠帶中，置於10±1℃之恆溫箱中保存，每隔七天定期取出接種。

二、培養基成份及培養環境

花葯培養之培養基以N_6 (Chu et al. 1975)培養基之無機鹽類及MS(Murashige and Skoog 1962)培養基之主要有機成份，加上1 mg / l 2,4-D,1 mg / l BA,0.2%活性碳，配合

不同濃度的蔗糖(3%,6%,9%及12%)及0.8%Sigma agar。花藥培養所誘導之胚狀體及癒合組織，再移至與花藥培養相同鹽類，加上15%椰子汁，0.2%活性碳，3%蔗糖及0.8%Sigma agar之培養基中(不含植物生長調節劑)進行植株誘導。花藥培養於25±1℃恆溫黑暗下，進行胚狀體之誘導。誘導出之胚狀體或癒合組織，移至分化培養基中，置於25±1℃恆溫，光照135μE / m²s，光期16小時的環境下培養。

結果與討論

圖1至圖5顯示麻竹花藥培養適當材料的選擇及穎花結構。圖1A花序之小穗過於幼嫩，小孢子時期尚未達到適合培養之成熟度，B花序之小穗外穎已張開消毒不易，只有C的花序為適合培養之材料，其小穗長度約1～1.2公分包裹緊密(圖2)。小穗剝去3～4層外穎後，便可顯露出小花(圖3)，小花之成熟度由外而內漸次成熟，花藥之顏色及大小亦由淡黃綠色漸次轉為黃色、由小而大(圖4)。除去內穎之小花顯露出含六個花藥及一個花柱之結構(圖5)。

由於花藥培養之母體植株感染霉菌，故消毒效果不佳，新鮮之材料約有百分之二十的發霉率，以10℃低溫處理兩週以上的材料，發霉率更高達95%以上。更高濃度或更長時間的消毒反而容易導致花藥白化死亡，而無助於減低發霉比率。因此若要降低發霉比率，除了改良消毒方法外，必須選擇本身健全未感染霉菌之植株。

接種技術影響麻竹花藥培養胚狀體形成與否甚鉅，一旦花藥受傷，受傷部位於幾天內即褐化萎縮，導致花藥死亡。未受傷的花藥平均於二至三週開始褐化，繼續培養約二個月左右可觀察到花藥壁裂開，形成肉眼可見之胚狀體或癒合組織(圖6)，一直到四個月左右才停止。而花藥壁及花絲等體細胞均無生長現象，切片證明胚狀體及癒合組織均源自小胞子(本文整理發表中)。花藥培養所產生質地緊密乳白色圓球形之擬胚化癒合組織(圖7)，在經過長達一年以上的繼代培養後，移至分化培養基中，仍能保有其分化能力(圖8)。胚狀體則能直接分化成單倍體植株(圖9)。但胚狀體及癒合組織常於分化成植株中途因嚴重褐化而死亡，猶待我們加以克服，以生產大量的單倍體植株。

花藥內小孢子發育時期是影響花藥培養能否成功的最主要因子之一。適當的培養時期依作物或品種不同而異(Chen 1977, Clapham 1971, Cornejo-Martin and Primo-Millo 1981, Genovesi and Collins 1982)。表1結果顯示小孢子發育時期決定麻竹花藥培養胚狀體之形成與否，含中單核期至早雙核期小孢子之花藥，胚狀體及癒合組織形成率較高。

培養基中最主要的有機成份為蔗糖，蔗糖主要的功用是供生長所需的碳源及調節滲透壓。一般而言花藥培養需要較高濃度的蔗糖(Chaleff and Stolarz 1981, Chen 1978)，在本研究所測試範圍中，麻竹花藥培養以9%之蔗糖濃度，誘導胚狀體及癒合組織形成的比

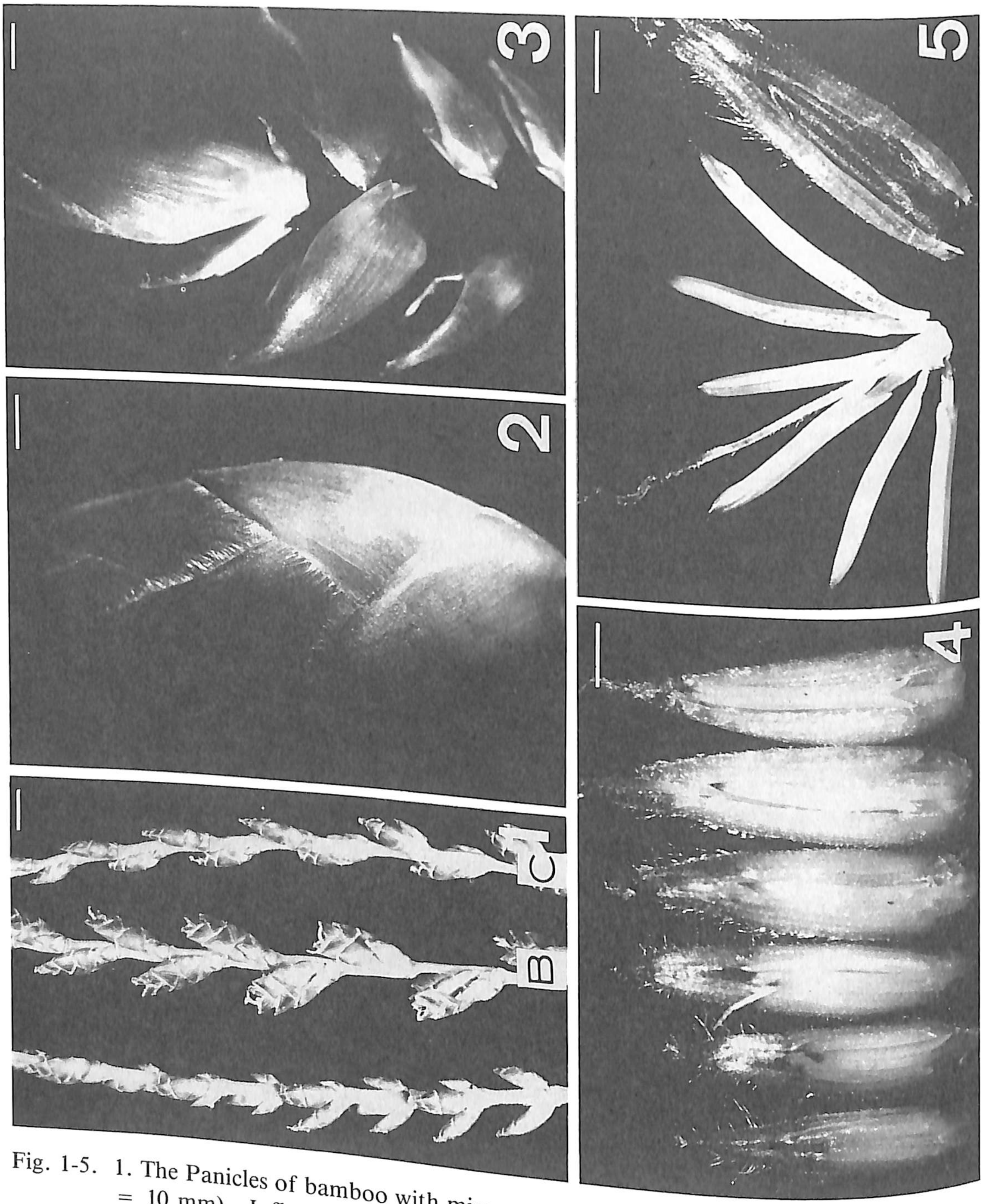

Fig. 1-5. 1. The Panicles of bamboo with microspores at different developmental stages (Bar = 10 mm). Inflorescence with microspores before uninucleate stage (A), at post-binucleate stage (B), and between uninucleate to early-binucleate stage (C). 2. Bamboo spikelets with anthers suitable for anther culture (Bar = 1 mm). 3. Floret exposed after 3-4 layers of glumes were removed (Bar = 1 mm). 4. Spikelet with 5-6 florets can be used as culture materials (Bar = 1 mm). 5. Each floret contains 6 anthers (Bar = 1 mm).

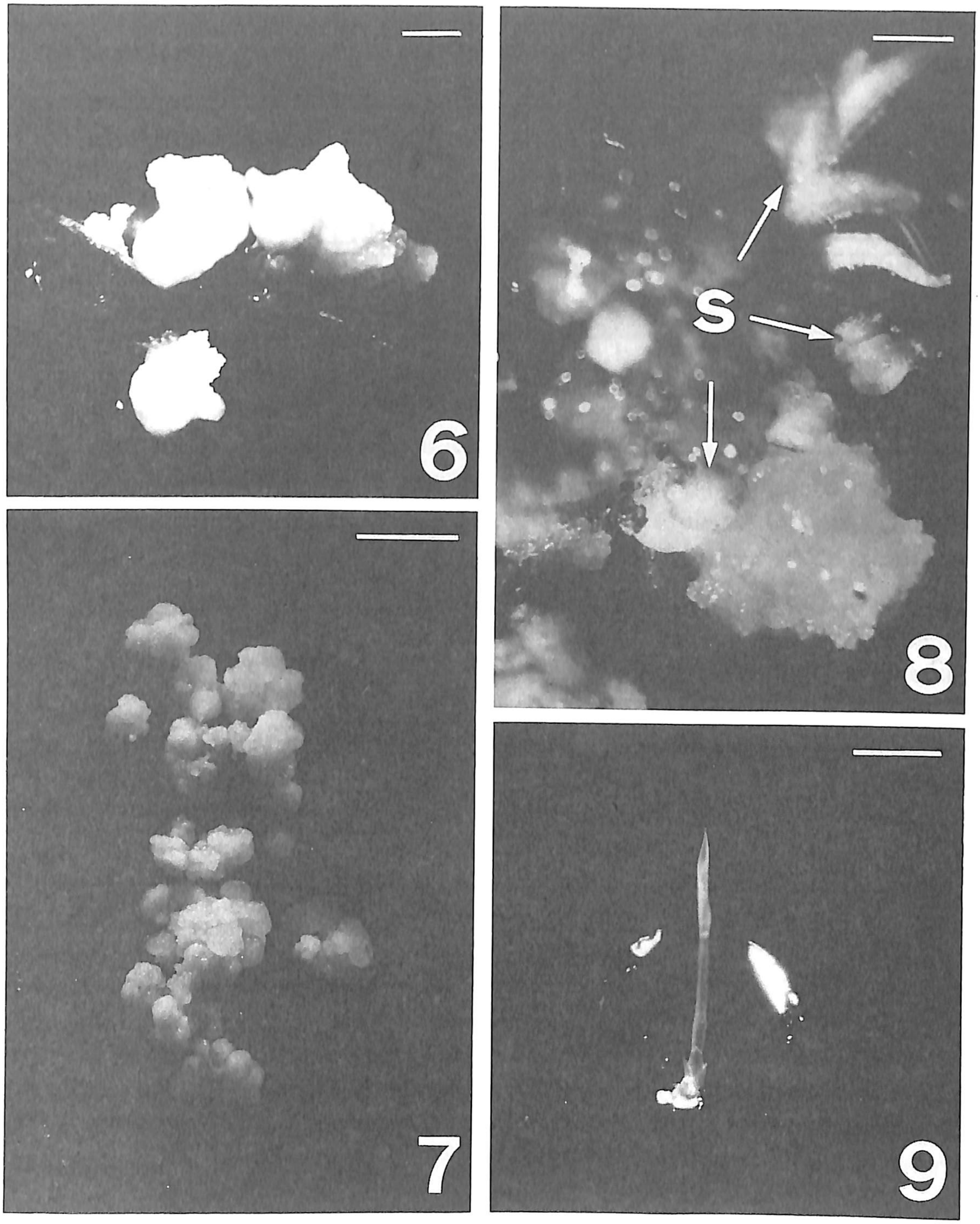

Fig. 6-9. 6. Pollen-derived embryoids from bamboo anthers cultured for two months (Bar = 1 mm). 7. Embryogenic callus induced from bamboo anther culture (Bar = 5 mm). 8. Clusters of embryoids protruding from the embryogenic callus and showing shoot (S) regeneration (Bar = 1 mm). 9. Haploid bamboo plant induced from anther culture (Bar = 10 mm).

Table 1. Influence of microspore developmental stage on the formation of embryoids in bamboo anther culture

Microspore developmental stage	No. of anthers cultured	Anthers producing embryoids and callus	
		Number	%
Tetrads	135	0	0.0 (0-3)
Early-uninucleate	1,070	9	0.8 (0-2)
Mid-uninucleate	1,540	57	3.7 (3-5)
Late-uninucleate	1,090	47	4.3 (3-5)
Early-binucleate	520	31	6.0 (4-9)

Basal medium contained N_6 inorganic salts and MS organic salts supplemented with 1 mg/l BA, 1 mg/l 2,4-D, 6% sucrose, 0.2% charcoal and 0.8% Sigma agar. Data in parentheses are the 95% confidence limits.

Table 2. Influence of sucrose concentration on the formation of embryoids in bamboo anther culture

Sucrose concentration (%)	No. of anthers cultured	Anthers producing embryoids and callus	
		Number	%
3	970	25	2.6 (1-3)
6	1,060	21	2.0 (1-3)
9	905	41	4.5 (3-5)
12	1,420	57	4.0 (3-5)

Anthers with microspores at early-uninucleate to early-binucleate stages were used. Basal medium was the same as shown in Table 1. Date in parentheses are the 95% confidence limits.

例最高(表 2)。

花葯培養前利用低溫進行花葯預措處理，以提高胚狀體或癒合組織的形成率，在許多作物已被廣範應用(Chen et al. 1982, Genovesi and Magill 1979, Tsay et al. 1988)。表 3 顯示麻竹花序於10℃低溫下預措一星期有助於提高胚狀體及癒合組織的形成率，但較長時

Table 3. Influence of cold (10°C) pretreatment of inflorescence before anther inoculation on the formation of embryoids in bamboo anther culture

Duration of cold treatment (days)	No. of anthers cultured	Anthers producing embryoids and callus	
		Number	%
0	1,338	49	3.7 (3- 5)
7	631	36	5.7 (3-10)

Microspore developmental stage of anthers cultured and basal medium composition were the same as in Table 2, except 9% instead of 6% sucrose was used. Data in parentheses are the 95% confidence limits.

Table 4. Influence of high temperature during culture on the formation of embryoids in bamboo anther culture

Anther source of donor plant	Treatment	No. of anthers cultured	Anthers producing embryoids and callus	
			Number	%
A	CK	1,435	49	3.4 (2-4)
	LT	1,158	78	6.7 (6-9)
	CK	1,359	4	0.3 (0-1)
B	LT	1,282	32	2.6 (2-4)
	AT	1,195	33	2.8 (2-4)

Microspore developmental stage of anthers cultured and basal medium composition were the same as in Table 2, except 9% instead of 6% sucrose was used. CK: cultured anthers were incubated at 25 ± 1°C; LT: cultured anthers were incubated at 25 ± 1°C for 1 month and transferred to 30° ± 1°C; AT: cultured anthers were incubated at 30 ± 1°C. Data in parentheses are the 95% confidence limits.

間的低溫處理，則因接種後材料發霉率過高，而無法獲得資料。

花葯來源母株的遺傳特性及生長情形能嚴重影響花葯培養之成敗(Yeh and Tsay 1987)。表4中種植於平地A株CK之誘導率爲3.4%，而種植於山區B株CK之誘導率僅0.3%，極可能因不同植株的遺傳特性不同或生長環境的差異，導至其胚狀體及癒合組織

形成比率差異達10倍以上。花藥培養環境亦爲決定培養成效的重要因子之一(Keller and Armstrong 1983, Yeh and Tsay 1988)。表4中30℃培養下的花葯，其胚狀體及癒合組織之形成較25℃提早約2～3週突破花葯壁。推測其原因可能爲較高溫度的培養可促使小孢子快速發育，而使胚狀體或癒合組織較早突破花葯壁，減少因褐化而死亡比率。

就禾本科而言，竹子被認爲是組織培養困難度較高的作物。因此有關竹子組織培養的文獻不多。Nadguda et al.(1990)利用*Bambusa arundinacea* willd及*Dendrocalamus brandisii* Kurz無菌播種小苗之節段培養，可促使竹子組織培養小苗提早開花且獲得種子，而有助於竹子生殖生長機制之研究及提高竹子育種之可能性。其它報告如種子無菌播種(Alexander and Rao 1968)，葉片組織原生質體的分離(Tseng et al. 1975)，及葉片、側芽培植體癒合組織的誘導(Huang and Murashige 1983)。在其它的培植體上亦有學者由誘導出之癒合組織成功的獲得再生的植株，如*Bambusa arundinacea* (Meta et al. 1982)，*Dendrocalamus strictus* (Rao et al. 1985)及*Sinocalamus latiflora* (Yeh and Chang 1987)以成熟種子爲材料，*Phyllostachys viridis* (Hassan and Debergh 1987)以嫩葉爲材料，*B oldhamii*、*B. beecheyana* (Yeh and Chang 1986a,b)以幼嫩花序爲材料，所誘導出之癒合組織均能經由體胚發生獲得再生植株。與上述諸位學者的研究比較，同樣的我們能在麻竹花葯癒合組織誘導培養基中獲得胚狀體及植株的再生。不同的是在培養基成份中的auxin，我們僅使用1mg／l 2,4-D，遠較其它報告所用的濃度爲低。且在培養基中0.2％活性碳的添加是必需的，否則花葯將嚴重褐化致使胚狀體或癒合組織無法順利成長。

花葯培養技術，已被廣範應用在許多禾本科作物的育種上。本研究麻竹花葯培養的初步成果，距離能實際應用尙有一段距離。欲達成此目標，必須往提高胚狀體及癒合組織形成率，克服胚狀體在分化成植株過程中因褐化死亡之阻礙，及發展單倍體植株倍加成同質雙倍體純系之技術等方向努力。

誌謝：文稿英文部份承蒙台灣省農業試驗所農藝系研究員劉大江博士斧正，特此申謝。本文爲台灣省農業試驗所研究報告第1519號。

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（收稿日期：79年10月11日；接受刊登日期：79年11月5日）